6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting and/or measuring and monitoring heptachlor and heptachlor epoxide in environmental media and in biological samples. The intent is not to provide an exhaustive list of analytical methods that could be used to detect and quantify heptachlor and heptachlor epoxide. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used to detect heptachlor and heptachlor epoxide in environmental samples are the methods approved by federal organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that refine previously used methods to obtain lower detection limits, and/or to improve accuracy and precision.

6.1 BIOLOGICAL MATERIALS

Analytical methods exist for measuring heptachlor, heptachlor epoxide, and/or their metabolites in various tissues (including adipose tissue), blood, human milk; urine, and feces. The common method used is gas chromatography (GC) coupled with electron capture detection (ECD) followed by identification using GC/mass spectrometty (MS). Since evidence indicates that heptachlor is metabolized to heptachlor epoxide in mammals, exposure to heptachlor is usually measured by determining levels of heptachlor epoxide in biological media. A summary of the detection methods used for various biological media is presented in Table 6-1.

Heptachlor and heptachlor epoxide are measured in adipose tissue, blood, and serum using GC/ECD (Adeshina and Todd 1990; Burse et al. 1990; Polishuk et al. 1977a, 1977b; Radomski et al. 1971a, 1971b) and identified by GC/MS (LeBel and Williams 1986). Sample preparation steps for adipose tissue vary but, in general, involve a lipid extraction step followed by a clean-up procedure involving gel permeation chromatography (GPC) and/or Florisil column clean-up. Using GPC with methylene chloride cyclohexane as a solvent, individual organochlorine contaminants can be separated from adipose tissue to produce extracts clean enough for direct GC analysis. Clean-up efficiency using GPC is 99.9% (LeBel and Williams 1986). The sensitivity obtained using GC/ECD is in the low-ppb range. Recoveries for heptachlor are adequate (72-87s); recoveries for heptachlor epoxide are good (84-98s). Precision is good for both (Adeshina and Todd 1990; LeBel and Williams 1986). The preparation step used for measuring heptachlor epoxide in blood and serum involves lipid extraction, clean-up with column chromatography, and elution with acetonitrile, hexane, and methylene chloride (Burse et al. 1990; Polishuk et al. 1977a, 1977b). Recovery is adequate (80-96s). Precision is good (9-l%). Sensitivity was not reported (Burse et al. 1990).

GC/ECD and GC equipped with a microcoulometric detector have been used to determine heptachlor and heptachlor epoxide in a variety of human tissues, including the liver, brain, adrenals, lungs, heart, kidneys, spleen, and pancreas (Curley et al. 1969; Klemmer et al. 1977; Radomski et al. 1968). Details of a sample preparation method were not reported for GC equipped with a microcoulometric detector (Curley et al. 1969). Sample preparation steps for GC/ECD include homogenization, extraction with petroleum ether or hexane, usually followed by a clean-up procedure (Klemmer et al. 1977; Radomski et al. 1968). Recovery, sensitivity, and precision data were not reported (Curley et al. 1969; Klemmer et al. 1977; Radomski et al. 1968).

Heptachlor and heptachlor epoxide have been measured in samples of human milk using GC/ECD and GC/MS (Mussalo-Rauhamaa et al. 1988; Polishuk et al. 1977b; Ritcey et al. 1972). Sample preparation

TABLE 6-1. Analytical Methods for Determining Heptachlor and Heptachlor Epoxide in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Adipose tissue	Lipid extraction with acetone-hexane; fractionation from fat by gel permeation chromatography; Florisil column clean-up.	GC/ECD; GC/MS	1.4 ng/g (heptachlor); 1.1 ng/g (heptachlor epoxide)	72–87% (heptachlor); 86–98% (heptachlor epoxide)	LeBel and Williams 1986
Adipose tissue	Lipid extraction with petroleum ether; concentration; clean-up on Florisil column.	GC/ECD	0.001 ppm (heptachlor epoxide)	84%	Adeshina and Todd 1990
Human liver and brain tissue	Grind liver tissue and extract with petroleum ether. Dry brain tissue and grind with petroleum ether. Centrifuge and inject.	GC/ECD	NR	NR	Radomski et al. 1968
Human tissues	Homogenize. Extract with hexane containing anhydrous sodium sulfate. Evaporate. Redissolve in hexane. Clean-up on Florisil.	GC/ECD	NR	NR	Klemmer et al. 1977

TABLE 6-1 (Continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood	Lipid extraction with chloroform/methanol; clean-up with column chromatography; elution with acetonitrile, hexane and methylene chloride.	GC/ECD	NR	NR	Polishuk et al. 1977a, 1977b
Serum	Add methanol and extract with hexane/ethyl ether. Clean-up on Florisil column. Acid treatment and clean-up on silica gel column.	GC/ECD	NR	80–96%	Burse et al. 1990
Human milk	Homogenize with chloroform/methanol; lipid extract with petroleum ether or hexane; clean-up by column chromatography; elution with acetonitrile, hexane, and methylene chloride.	GC/ECD	NR	NR	Polishuk et al. 1977b
Human milk	Lipid extraction with acetone-hexane. Dissolve in benzene-acetone. Clean-up on Florisil. Elute with dichloromethane-petroleum ether. Concentrate and add hexane	GC/ECD	0.001 ppm (heptachlor epoxide)	NR	Ritcey et al. 1972

TABLE 6-1 (Continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine and feces (heptachlor, heptachlor epoxide, and metabolites)	Extract with acetone and hexane. Combine solvents and concentrate. Mix with silicic acid and air dry. Clean-up on Florisil column and silicic acid column. Metabolites extracted into hexane for GC analysis.	GC/ECD	NR	NR	Tashiro and Matsumura 1978

ECD = electron capture detector; GC = gas chromatography; MS = mass spectrometry; NR = not reported

steps for milk involve homogenization with chloroform/methanol, lipid extraction with petroleum ether, hexane or acetone-hexane, clean-up by column chromatography, and elution with acetonitrile, hexane, methylene chloride, or dichloromethane-petroleum ether. Precision, accuracy, and sensitivity were not reported for most of the studies; however, one study reported a sensitivity in the low-ppb range (Ritcey et al. 1972).

Heptachlor, heptachlor epoxide, and their metabolites have been measured in urine and feces using GC/ECD (Tashiro and Matsumura 1978). Sample preparation steps involve extraction with acetone and hexane, clean-up on Florisil and silicic acid columns, and extraction of the derivatized metabolites into hexane for GLC analysis. Precision, accuracy, and sensitivity were not reported (Tashiro and Matsumura 1978).

6.2 ENVIRONMENTAL SAMPLES

Methods exist for measuring heptachlor and heptachlor epoxide in air, water, soil, and food. The most common methods are GC/ECD and GC/MS. A summary of methods for detecting heptachlor and heptachlor epoxide in various environmental samples is presented in Table 6-2.

Heptachlor is measured in indoor and outdoor air samples using GC/ECD and GC/MS (Anderson and Hites 1989; Lewis et al. 1986; NIOSH 1979; Savage 1989). Heptachlor has also been measured in house dust (Roberts and Camann 1989). Preparation methods involve the use of a variety of air trapping samplers. Examples of these include the Greenburg-Smith impinger, Chromosorb 102, low-volume samplers, and the Millipore miniature vacuum pump with a sampling tube. The next step includes extraction with diethyl ether, acetone-hexane, or toluene (Anderson and Hites 1989; NIOSH 1989; Roberts and Camann 1989). For indoor air, precision is excellent and recovery is adequate (>75%). Sensitivity is in the sub-ppb range (NIOSH 1979). For outdoor air, precision is good (13%) and recovery is excellent (99%). Sensitivity is in the sub-ppb range (Lewis et al. 1986).

Heptachlor and heptachlor epoxide are measured in water, drinking water, waste water, soil/sediment, and solid waste using GC/ECD and GC/MS (Alford-Stevens et al. 1988; EPA 1986d; Lopez-Avila et al. 1990; McDougall et al. 1987; Smith et al. 1987). Preparation of water, waste water, and drinking water samples involves extraction with methylene-chloride, concentration, and solvent exchange to hexane or methyl *tert*-butyl ether. Mean recovery in water for heptachlor was low (52-68%) and precision was poor (48-57%) (Alford-Stevens et al. 1988). Poor recovery and precision data were thought to be attributable to chromatographic problems in some of the participating laboratories. For drinking water (EPA Method 508), recovery was excellent for heptachlor (99%) and heptachlor epoxide (95%). Precision was excellent for both compounds (<10%). Sensitivity was in the sub-ppb range (Lopez-Avila et al. 1990). Preparation of soil/sediment or solid waste samples involves extraction with methylene chloride, methylene chloride-acetone, methylene chloride-methanol, or acetone-hexane followed by clean-up with Florisil or GPC (Alford-Stevens et al. 1988, EPA 19864). Overall precision was adequate to poor, ranging from 19% to 47% for heptachlor. Recovery and sensitivity were not reported (Alford-Stevens et al. 1988). EPA Test Methods 8080 and 8250 for evaluating waste water, soil sediment, and solid waste report sensitivity in the low-ppb range for both heptachlor and heptachlor epoxide (EPA 1986d). Recovery for heptachlor is adequate (69-87%) and recovery for heptachlor epoxide is good (89-92%). Precision is adequate for both methods (EPA 1986d).

GC/ECD is the method used to detect heptachlor and heptachlor epoxide in foods (butterfat, fruits, vegetables, milk, and animal feed) (Di Muccio et al. 1988, Hopper and Griffitt 1987; Korfmacher et al.

TABLE 6-2. Analytical Methods for Determining Heptachlor and Heptachlor Epoxide in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Outdoor air	Sample collected with low-volume sampler consisting of a constant flow pump and a cartridge containing polyurethane foam. Extract with diethylether in hexane.	GC/ECD; GC/MS	0.0006 ppb	99% (heptachlor)	Lewis et al. 1986
Indoor air	Sample collected through a glass tube containing Chromosorb 102. Desorption with toluene.	GC/ECD	0.1 ppb	>75% (heptachlor)	NIOSH 1979
House dust	Sample collected with high-volume surface sampler; extract with diethyl ether in hexane.	GC/ECD; GC/MS	NR	NR (heptachlor)	Roberts and Camann 1989
Water	Extract with methylene chloride.	GC/MS	NR	52–68% (heptachlor)	Alford-Stevens et al. 1988
Wastewater	Extract with methylene chloride; exchange to hexane.	GC/ECD (EPA Method 8080)	0.003 μg/L (heptachlor); 0.083 μg/L (heptachlor epoxide)	69% (heptachlor); 89% (heptachlor epoxide)	EPA 1986d
Wastewater	Extract with methylene chloride	GC/MS (EPA Method 8250)	1.9 μg/L (heptachlor); 2.2 μg/L (heptachlor epoxide)	87% (heptachlor); 92% (heptachlor epoxide)	EPA 1986d

TABLE 6-2 (Continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Drinking water	Extract with methylene chloride; solvent exchange to methyl tert-butyl ether.	GC/ECD (EPA Method 508)	0.01 μg/L (heptachlor); 0.015 μg/L (heptachlor epoxide)	99% (heptachlor); 95% (heptachlor epoxide)	Lopez-Avila et al. 1990
Soil/ sediment and solid waste	Extract with methylene chloride; clean-up extract.	GC/MS (EPA Method 8250)	1.9 μg/L (heptachlor); 2.2 μg/L (heptachlor epoxide)	87% (heptachlor); 92% (heptachlor epoxide)	EPA 1986d
Foodstuff (butterfat)	Lipid extraction with automated gel permeation chromatography; direct injection.	GC/ECD	NR	100% (heptachlor epoxide)	Hopper and Griffitt 1987
Milk	Extract on solid-matrix disposable columns by means of acetonitrile-saturated light petroleum; Florisil® clean-up.	GC/ECD	NR	99% (heptachlor epoxide)	DiMuccio et al. 1988

ECD = electron capture detector; EPA = Environmental Protection Agency; GC = gas chromatography; MS = mass spectrometry; NR = not reported

1987; Ober et al. 1987; Santa Maria et al. 1986). Preparation methods vary for the different types of foods. The sample preparation method for butterfat involves GPC. GPC is a rapid clean-up technique for separating pesticide residues from a lipid extract. It was developed into an automated clean-up apparatus for use on a wide variety of fats and oils. The automated GPC system is reproducible and reliable. After being cleaned on GPC, most samples can be analyzed by GC without additional clean-up (Hopper and Griffitt 1987). Recovery is complete (l00%), and precision is very good (<3%). Sensitivity is in the subppm range. The sample preparation for milk samples involves selective extraction on solid-matrix disposable columns by means of acetonitrile-saturated light petroleum, followed by Florisil column clean-up. Recovery is excellent (99%); precision is very good (<7%) (Di Muccio et al. 1988). Sample preparation for fruits, vegetables, and animal feed involves cyclic steam distillation extraction in hexane or isooctane with direct injection into the gas chromatograph. Recoveries for this method are very low (15~50%). This is an indication that heptachlor is not extracted quantitatively by steam distillation and is not a recommended preparation method (Ober et al. 1987; Santa Maria et al. 1986).

6.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of heptachlor and heptachlor epoxide is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of heptachlor and heptachlor epoxide.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce or eliminate the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. Methods exist for determining levels of heptachlor,, heptachlor epoxide, and/or their metabolites in various tissues (including adipose tissues) (Adeshina and Todd 1990; Curley et al. 1969; Klemmer et al. 1977; LeBel and Williams 1986; Radomski et al. 1968), milk (Mussalo-Rauhamaa et al. 1988; Polishuk et al. 1977b; Ritcey et al. 1972), blood (Polishuk et al. l977a, 1977b), serum (Burse et al. 1990), urine, and feces (Tashiro and Matsumura 1978). Methods for determining levels in adipose tissue are sensitive for measuring levels at which health effects might occur as well as background levels in the population. Methods for determining heptachlor and heptachlor epoxide in adipose tissue are relatively precise. Recovery is better for heptachlor epoxide than for heptachlor. Data on the determination of heptachlor and heptachlor epoxide in tissues, blood, serum, milk, urine, and feces are limited as precision, recovery, and/or sensitivity data were not reported for the existing methods. More information on the precision, accuracy, and sensitivity of these methods is needed to evaluate the value of using levels of heptachlor and heptachlor epoxide as biomarkers of exposure. :

The methods for determining biomarkers of effect are the same as those for exposure and are subject to the same limitations. Improved methods could allow a better assessment of the relationship between levels

of heptachlor and heptachlor epoxide in body tissues, blood, and fluids and the known health effects associated with these chemicals.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Existing methods for determining levels of heptachlor in air are sensitive enough to measure background levels in the environment, as well as levels at which health effects might occur. Data on the determination of heptachlor and heptachlor epoxide in air (Anderson and Hites 1989; Lewis et al. 1986; NIOSH 1979; Roberts and Camann 1989; Savage 1989), water (Alford-Stevens et al. 1988; EPA 1986d; Lopez-Avila et al. 1990), soil (EPA 1986d; McDougall et al. 1987; Smith et al. 1987), and food (Di Muccio et al. 1988; Hopper and Griffitt 1987; Korfmacher et al. 1987; Ober et al. 1987; Santa Maria et al. 1986) are limited. Information on the accuracy, precision, and sensitivity of these methods would permit better assessment of the risk of low-level environmental exposure for these media. A preparation method for fruit and vegetable analysis that provides increased recovery would allow better assessment of the risk of dietary exposure. Research investigating the relationship between levels measured in air, water, soil, and food and observed health effects could increase our confidence in existing methods and/or indicate where improvements are needed.

6.3.2 On-going Studies

No on-going studies regarding analytical methods were located for heptachlor or heptachlor epoxide.